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ScienceDirect
Applied Animal Behaviour Science 114 (2008) 132–148
APPLIED ANIMAL BEHAVIOUR SCIENCE
www.elsevier.com/locate/applanim

Social instability increases aggression in groups of dairy goats, but with minor consequences for the goats' growth, kid production and development

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Accepted 7 January 2008
Available online 21 February 2008

Abstract

The aim of the present study was to study the effects of social instability (regrouping) during the second trimester (7 weeks) of pregnancy on aggression, cortisol concentrations and growth in goats and its consequences for survival, growth and some aspects of behavioural development in the kids. Six weeks after mating, 32 goats were distributed into eight groups. In four of the groups, randomly chosen pairs of goats were rotated between groups every Monday morning (around 08:30 h) for 7 weeks (unstable groups), starting 6 weeks into gestation (second trimester). The remaining four groups were kept stable throughout the entire pregnancy (stable groups). The adult goats were video recorded for 6 h twice a week for the first, second, fourth and seventh regrouping and for the 2 last weeks before expected birth. Blood samples of the adult goats were collected in a period from 1 week before the start of the first regrouping and until 1 week before expected birth. Blood samples from a maximum of two kids from each litter were collected at 3 weeks of age. Two kids from each litter were subjected to two types of behavioural tests: a 'social test' at the age of 1 and 7 weeks and a 'novel object test' at the age of 5 weeks. Except for the higher aggression level in

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doi:10.1016/j.applanim.2008.01.007

the unstable groups, social instability did not have any other significant effects on factors such as growth, cortisol level or kid production in the goats. There were only minor effects on the behavioural development in the goat kids. However, kids from the unstable groups showed more escape attempts in the first trial of a 'social test', spent more time in contact with a novel object and unfamiliar kids, and showed less fear (escape attempts) after they were given one exposure to the social test situation. Furthermore, kids from unstable groups had a lower basal cortisol level than kids from stable groups.

Keywords: Agonistic behaviour; Cortisol; Dairy goats; Goat kids; Maternal effects

1. Introduction

Like other farm animals, the domestic goat may react aversively to unfamiliar individuals, and aggression can be minimized by avoiding the introduction of new individuals into established groups. High levels of chasing, fighting and displacement from resources may indicate social stress in a group of animals (e.g. pigs: Andersen et al., 1999; sheep: Bøe et al., 2006; goats: Jørgensen et al., 2006). Possible consequences are lower access to food, water and attractive lying areas and reduced time for rest (Andersen et al., 1999; Andersen and Bøe, 2007; Bøe et al., 2006). This might result in lower feed intake and a lower growth (Stookey and Gonyou, 1994), suppressed immune response (Tuchscherer et al., 1998; De Groot et al., 2001), reduced milk yield (Hasegawa et al., 1997; Fernández et al., 2007) and lower reproductive success (e.g. Mendl et al., 1992; Bakken, 1993a,b).

Although aggression was enhanced for about 1 or 2 days after every regrouping in French Alpine goats, a significant decrease in milk production was only seen after the first regrouping (Fernández et al., 2007). A similar study in heifers indicated that repeated regrouping consistently increased the amount of agonistic interactions and that this persisted through several regrouping events (Raussi et al., 2005). However, recent reports in sows did not reveal any clear relationship between aggression after mixing and reproductive ability (Jarvis et al., 2006; Kongsted, 2006), suggesting that farm animals may be surprisingly robust even when subjected to intense aggressive interactions. Female mountain goats, the ancestor of our domestic goat, are reported to interact aggressively much more frequently than other female ungulates (reviewed by Fournier and Festa-Bianchet, 1995). This high level of aggressiveness may be due to an evolutionary adaptation to a rough home range environment characterised by rigid and rocky terrain with scarce food resources and where a high competitive ability is an advantage. The social behaviour of goats is fairly well described (e.g. Shackleton and Shank, 1984; Barroso et al., 2000; Fournier and Festa-Bianchet, 1995; Côté and Festa-Bianchet, 2001). However, the consequences for welfare and production of the repeated regrouping that is routinely practised in commercial herds are not well known.

Kaiser and Sachser (2005) argue that in evolutionary terms, mothers should try to maximise fitness by adjusting the phenotype of their offspring to the environment in which they live. Most applied studies on domestic species assume that there should be negative effects of prenatal stress. In contrast, evolutionary biologists would not at all use the term 'prenatal stress', but rather discuss maternal effects, assuming that the best strategy is to control the offspring's development to fit the current environmental conditions. However, extreme stress which is far beyond the animals' ability to cope could also produce mal-adaptive or pathological effects. In high-density populations of *Cavia aperea*, the feral ancestor of the domestic guinea pig, the offspring will become more offensive and competitive, whereas the opposite may occur in periods with low population density (e.g. Rood, 1972; Asher et al., 2004). This is of particular relevance to farm animals since high-density, unstable populations are the norm in our production environments. We may in fact speculate whether farm animals are indirectly selected to have a strong ability to cope with social stress. Prenatally stressed lambs with mothers subjected to an isolation treatment, showed more exploration (Roussel et al., 2008) and locomotion (Roussel et al., 2004) in a novel environment than control lambs. Similar results were found in blue fox cubs subjected to prenatal handling stress (e.g. Braastad, 1998; Braastad et al., 1998). Increased exploration followed by more jumps, illustrating an attempt to actively escape the novel situation is also documented for some species (e.g. rodents: Barbazanges et al., 1996; goat kids: Roussel et al., 2005). These results may seem surprising if only negative effects of prenatal stress are expected, but in terms of the 'environment-adaptation hypothesis' they all make sense in that the offspring would benefit from being alert, explorative and flexible in a stressful environment. Since obtaining information by exploring is an essential feature of an animal's survival (Carson, 1985), the animal should choose the most efficient behavioural strategy for achieving this goal.

The overall aim of this experiment was to investigate the effects of social instability (regrouping) during 7 weeks of pregnancy on aggression, cortisol concentrations and growth in goats and its consequences for survival, growth and some aspects of behavioural development in the kids. We predicted that social instability in groups of adult, female goats would induce higher aggression levels, especially shortly after regrouping, and that this would be associated with higher plasma cortisol concentrations and a lower growth than in goats that were allowed to stay in the same social group throughout pregnancy. Following the 'environment-adaptation' hypothesis, we may predict that kids from mothers in the unstable social groups would more actively explore the novel stimuli (novel object and unfamiliar kids) and show more escape attempts when being isolated in these test situations than kids from the control mothers.

2. Material and methods

2.1. Experimental set-up

Six weeks after mating, 32 goats were distributed into eight groups (four goats in each), subjected to either of two different treatments; stable or unstable social groups. In four of the groups, the goats remained in the same group throughout the entire pregnancy (not mixed with new individuals; stable groups). In the other 4 groups, randomly chosen pairs of goats (the same pair each week) were rotated between groups every Monday morning (around 08:30 h) for 7 weeks of pregnancy (unstable groups), starting at 6 weeks of gestation (in the second trimester of pregnancy). The rotation scheme resulted in each pair in the unstable treatment having to meet two new pairs of goats and to change pen each week. From the time of last regrouping until weaning of the kids at 6 weeks of age, the goats from the unstable groups were not moved or regrouped. The reason for choosing to rotate pairs instead of single goats was that we wanted to avoid overt aggression towards only one individual. The selection criteria for the initial regrouping both for the unstable and stable groups were that there should be a maximum variation in weight within each group, that there should be no significant difference in weight between the two treatments (stable vs. unstable groups), and that the males to which the goats were naturally mated were represented equally in the two treatments.

2.2. Animals, housing and feeding

Healthy, pregnant (not returning to oestrus during the 6 weeks from mating until the start of the experiment and/or checked with ultra-sound) Norwegian dairy goats, aged 4.0 ± 0.3 years (range 2–9 years) and weighing on average 60.5 ± 1.4 kg (range 44.0–74.5) were used in the experiment. We chose goats that were expected to give birth within the same week to ensure that they were in the same gestation phase throughout the entire experimental period. The goats were individually marked.

From the time when the goats were transported from pasture (late August/early September) until mating (late September/early October), they were kept in a large, female group and housed in an insulated, mechanically ventilated room with a room temperature of approximately 10 °C and expanded metal flooring. No males were present in the group. They were kept inside during feeding in the morning (around 08:00 h) and from feeding in the afternoon (at around 14:30 h) until the next morning feeding. During the rest of the day, they were kept in an outside rocky enclosure with access to grass and trees and an uninsulated room with straw bedding and free access to hay. The goats were fed 0.1 kg of concentrates every morning for most of the period, but this was gradually increased up to 0.5 kg in the last part of pregnancy from the beginning of January until the end of February. They had free access to grass silage supplied every morning and afternoon, except for 1 day each week when silage was replaced by hay to stimulate the goats' digestion.

At the start of the experiment, 6 weeks after mating, the experimental goats were grouped according to the above mentioned criteria and placed in a separate, rectangular pens (2.70 m × 3.25 m, 2.20 m^2 per goat) and expanded metal flooring in most of the pen except from a 60 cm deep area made of solid wood at the rear end of the pen. The pen walls were made of 1.5 m high solid, water-resistant, 15 mm plywood to avoid physical contact between groups (pens). All pens were located in an insulated and mechanically ventilated room with a room temperature of approximately 10 °C. One drinking bowl allowing free access to water was located in the rear end of all pens, and the front part of each pen included four eating places (one for each goat) with access to a common feeding trough and a door. The pens were cleaned immediately after morning and afternoon feeding and new bedding was added to the solid floor area. In addition to natural light through windows on both sides of the room, artificial light was kept on between 08:00 and 15:00 h.

At the time of expected birth, the experimental pens were divided into four equal squares with solid, wooden walls, and each goat and her newborn kids were kept isolated from the rest of the group for 2 days. This was done to make them bond properly with their offspring. Afterwards, the extra walls were removed, and the four goats and their kids were kept together as a group. The openings in the front part of the pen allowed all kids to go back and forth between their home pen and a kid area that was supplied with a wooden floor and free access to hay.

2.3. Blood sampling of goats and kids

Blood samples were collected from the jugular vein into heparinized tubes using a Vacutainer® (Becton and Dickinson, Leuven, Belgium). Blood samples were drawn at around 08:30 h every Tuesday (day 2; the day after rotation and video-recording behaviour of the goats) and Thursday (day 4; the day before video-recording behaviour of the goats) in a period from 1 week before the start of the first rotation (two control samples; week 0) and until 1 week before expected birth. These days were chosen to avoid interfering with behaviour during video recording. During blood sampling, the goat stood between the legs of a familiar, trained stockperson that held it gently with one hand on the chest and the other placed under the chin, while another familiar, trained research technician collected the sample. The whole procedure took around one minute per goat. In a similar way as the adult goats, blood samples were drawn at 3 weeks of age (09:00 h) from a maximum of two kids (one sample per kid; if possible one male and one female) from each litter. Cortisol was measured in a total of 50 kids, of which 21 were from stable groups and 29 were from unstable groups.

Plasma was separated by centrifugation at 3000 × g for 15 min within 2 h. The plasma was stored at −20 °C until it was thawed and analysed for cortisol. Cortisol was measured by radioimmunoassay according to Simensen et al. (1978) with the following modifications: 20 μl plasma was boiled in 500 μl of 0.75% trichloroacetic acid (TCA) and 0.225% NaOH for 10 min. After adding antiserum and ^{3}H-cortisol-mixture, the samples were incubated for 1 h at room temperature, then overnight at 4 °C. Phosphate buffer used for incubation contained 0.2% bovine serum albumin (BSA). Antiserum (F3-314) was obtained from Esoterix Endocrinology, Calabasas Hills, CA, USA. The assay was validated for use with goat plasma by demonstrating parallelism between dilutions of plasma samples and the standard curve. The range of the standard curve was 3.1–50 ng/ml. Assay sensitivity was 2 ng/ml, corresponding to 95% binding of the labelled hormone. The inter-assay coefficients of variation for samples with 4.85, 16.9 and 41.5 ng/ml cortisol were 7.2, 6.5 and 7.0%, respectively ($n = 26$).

2.4. Weighing and reproduction data

All the goats were weighed 2 days prior to the start of the experiment and immediately after the seventh-week of regrouping. The goat kids were weighed at birth (unstable: $n = 34$, stable: $n = 26$) and weaning (unstable: $n = 28$, stable: $n = 23$). The number of live born kids, the number of each sex, and kid survival were recorded for each litter. One kid from litters with three siblings was taken out of the experiment and offered automatic milk feeding.

2.5. Behavioural observations

The adult goats were video recorded for 6 h from around 08:30 h every Monday (day of regrouping) and Friday for the first, second, fourth and seventh regrouping and for the 2 last weeks before expected birth. One camera was suspended over each pen and directly connected to a computer with the MSH digital video system (http://www.guard.lv).

We used the following ethogram of mutually exclusive agonistic behaviours, which is a modification of previous ethograms used in similar goat studies (e.g. Shank, 1972; Andersen and Bøe, 2007):

- Nuzzling/exploring (nose in contact with) another goat;
- frontal clashing (a behaviour where one or both individuals rear onto their hind legs with the head and torso twisted and then forcefully descends onto the front legs delivering a powerful blow forwards and downwards against the head of the receiver) or butting with the head towards the head or shoulders of another goat;
- butting with the head towards other parts of the receivers' body;
- chasing (quickly following) another goat that tries to escape;
- threatening (pawing or rushing towards, or directing the forehead towards the opponent but without physical contact);
- avoiding (moving the head and/or body away from an approaching goat, but with no physical interaction);
- withdrawing (moving the head and/or body away from another goat after a social interaction);
- actively displacing (physically forcing another goat to leave its resting position or feeding place by pushing or butting sideward or from behind);
- passively displacing (making another goat leave its resting position or feeding place simply by approaching that individual, but without any physical contact).

In the analysis frontal clashing and butting towards the head and the rest of the body were summed into 'clashing/butting activity', while chasing, threatening, and displacing were summed into 'other offensive behaviours'. Finally, avoiding and withdrawing were pooled into 'defensive behaviours.

2.6. Behavioural tests

A maximum of two kids from each litter (if possible one male and one female) were subjected to two types of behavioural tests: a 'social test' at the age of 1 and 7 weeks (1 week after weaning) and a 'novel object test' at the age of 5 weeks. Both tests were conducted in the home pen to make sure that it was only the social kid stimulus and the novel object that were unfamiliar to the test animal (Fig. 1). During the 5 min that each test lasted, the test kid was isolated from the rest of the group, including its mother. The home pen was split into two compartments with a solid wooden wall of the same height and type as the pen walls to make a relatively long and narrow test arena in the front part of the pen. The floor in the test arena was then divided into three equal areas that were marked: area 1 where the test kid always entered, area 3 where the social

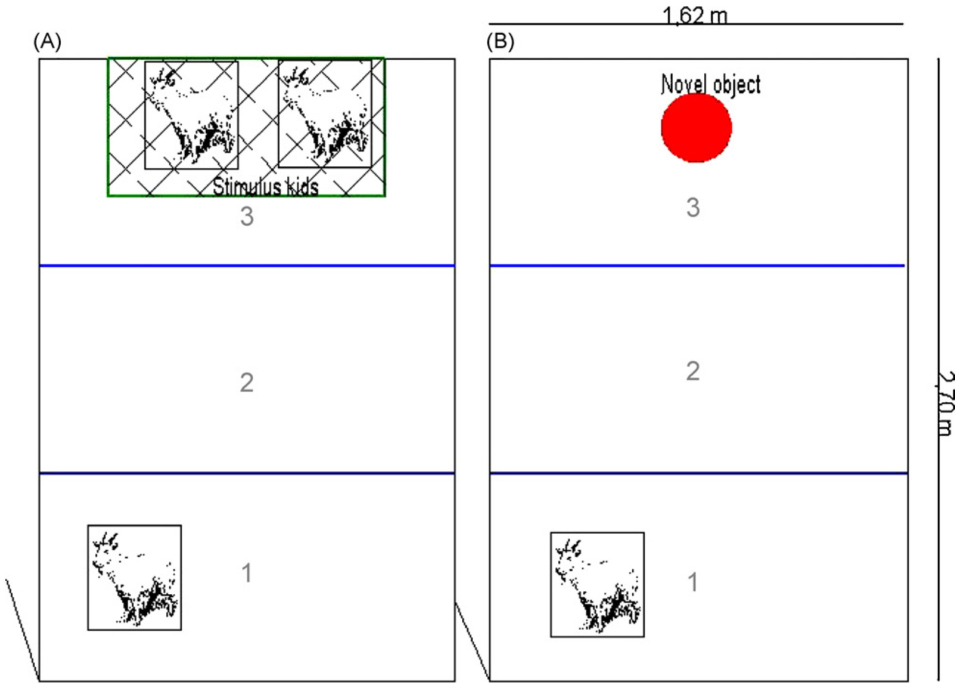

Fig. 1. A schematic picture of the test arena comprising half of the goats' home pen: (A) depicts the social test including a cage with the two stimulus kids and (B) depicts the test with the novel object.

stimulus or the novel object was placed, and area 2 in between (Fig. 1). The kids were tested in a balanced order.

2.6.1. The 'social test'

The 'social test' was a modification of a test used by Boivin and Braastad (1996) for goat kids and by Færevik et al. (2006) for young calves. During the test, the kids were subjected to two unfamiliar stimulus kids (not experimental subjects, but of the same age) placed in a cage with straw bedding located in the test arena/home pen (Fig. 1A). The cage allowed both visual and nose contact. Shortly before testing, the entire group consisting of four goats and their kids was transported to another room, and the kids were placed in a transport trolley in front of the entrance of the experimental room to minimize pre-test handling. Each kid was gently lifted out of the trolley and put into the experimental pen through the entrance door in area 1, which was in the side of the test arena opposite to the stimulus cage. The following behavioural responses were scored directly by an observer standing outside the pen, but out of sight of the test subject, and by another observer watching the computer screen directly:

- latency (in seconds) to enter the area with the stimulus cage (area 3);
- latency (in seconds) to nose contact with the stimulus cage;
- time spent (in seconds) in area 3;
- time spent (in seconds) nosing at the cage.

The behaviour of the kids during this test was also video recorded, and the following variables were scored from video:

- no. of escape attempts (when the kid ran towards one of the pen walls and/or jumped towards it);
- no. of times the kid nosed the pen fittings.

2.6.2. The 'novel object test'

Novel object tests have been successively used both on lambs (e.g. Roussel et al., 2004) and goat kids (e.g. Roussel et al., 2005), but usually in a novel test arena. We chose the home pen as the test arena in our experiment to increase the specificity of the test. Before the experiment, the goats and their kids were transported and handled in exactly the same way as described in the 'social test'. The novel object was a 10 l red plastic bucket which was placed upside down in the middle of area 3 of the test arena. As for the 'social test', the test kids always entered in area 1.

The following behavioural responses were scored through direct observations:

- latency (in seconds) to enter area 3;
- latency (in seconds) to nose contact with the novel object;
- time spent (in seconds) in area 3;
- time spent (in seconds) nosing at the novel object;
- no. of vocalisations.

The following variables were scored from the video:

- no. of escape attempts (when the kid ran towards one of the pen walls and/or jumping towards it);
- no. of times the kid nosed the pen fittings.

2.7. Statistics

Cortisol measures and agonistic behaviour in each experimental week were analysed using an analysis of variance model (GLM procedure in SAS, Hatcher and Stepanski, 1994), with stable versus unstable groups as the main treatment (class variable), day (class variable), the interaction between the main treatment and day (class variable), and weight and age of the goats as continuous variables. Differences in the level of agonistic interactions between the days of regrouping were analysed within treatment by using a one-way analysis of variance model with day/date as class variable (GLM procedure). Growth of the goats during the experimental period was analysed by using a general linear model including treatment as class variable and age as a continuous variable. Birth weight, growth and cortisol level of the kids were analysed using a general mixed linear model, including treatment, sex and litter size as fixed class effects, and with goat as a random class effect.

We analysed the treatment effects on litter size and sex ratio using the Genmod procedure in SAS (SAS Institute Inc., 1989). This procedure fits generalized linear models for non-normally distributed data through the use of non-linear link functions. In the analyses of litter size and sex ratio, multinomial distributions of the dependent variables were assumed and the cumulative logit link ("cumlogit") was thus used. Treatment and age class of goats (2 years old, 3–4 years old, and $\geq$5 years old) were entered as categorical effects, and weight was included as a covariate.

In the analysis of treatment effects on sex ratio, only twin litters (22 of the 28 litters) were included to reduce the number of litter type classes, and the response variable for sex ratio was defined as "litter type" (FF = two females; FM = one male + one female; MM = two males). The response variable for litter size was one, two or three kids.

The kids' responses during the behavioural tests were analysed using a general linear model (GLM procedure in SAS) with treatment and sex as class variables. Pearson correlation analysis was used to test the relationship between mean cortisol concentrations in the experimental period and 1 week before expected delivery and between mean cortisol concentration and number of live born kids.

3. Results

3.1. Cortisol measures in the adult goats

During the course of the study the mean cortisol concentrations varied between 2 and 10 ng/ml. Basal level of cortisol measured before the goats were put into the experimental pens and cortisol measured at the time of first regrouping did not differ significantly between the two treatments. There was no effect of treatment on the level of cortisol at any time of the experimental period, and the stable groups followed the same pattern of variation in cortisol throughout the entire experimental period as the unstable groups (Fig. 2). The cortisol level declined to a much lower level shortly before birth in both treatment groups. Weight and age of the goats did not affect the cortisol concentration.

3.2. Agonistic behaviour during the treatment period

At the time of first regrouping (week 1) there were no significant differences in the amount of agonistic interactions between the two treatments (Figs. 3 and 4), but the goats were more frequently involved in butting/clashing activity on day 1 than 5 ($F_{1,58} = 6.9$, $P < 0.05$). The frequency of agonistic behaviours were significantly higher both at the time of second (week 2), fourth (week 4) and seventh (week 7) regrouping in the unstable groups than in the other four groups that were allowed to remain stable throughout the entire period (Figs. 3 and 4; (2) regrouping: butting and clashing: $F_{1,58} = 5.4$, $P < 0.05$; other offensive and defensive behaviours: ns; (4) regrouping: butting and clashing: $F_{1,58} = 5.0$, $P < 0.05$, other offensive behaviours: $F_{1,58} = 4.0$, $P < 0.05$; defensive behaviours: $F_{1,58} = 5.8$, $P < 0.05$; (7) regrouping: butting and clashing: $F_{1,58} = 7.0$, $P < 0.05$; other offensive behaviours: $F_{1,58} = 2.9$, $P = 0.09$; defensive behaviours: $F_{1,58} = 4.2$, $P < 0.05$). The number of agonistic behaviours always declined from day 1 to day 5 after regrouping (Figs. 3 and 4), and this was significant for butting/clashing in week 1 ($F_{1,58} = 6.9$, $P < 0.05$), week 2 ($F_{1,58} = 11.2$, $P < 0.01$) and week 4

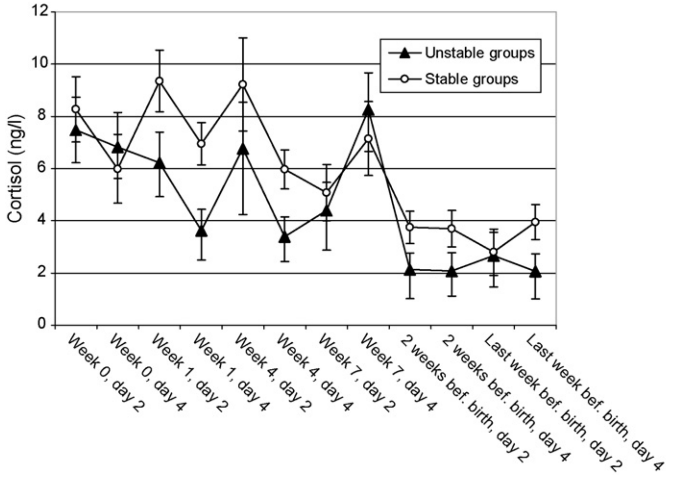

Fig. 2. Variation over time in the blood plasma cortisol content (means ± S.E.) of goats in unstable and stable treatment groups. Day 2 is the day after rotation and first video recording whereas day 4 is 4 days after regrouping and the day before video recording.

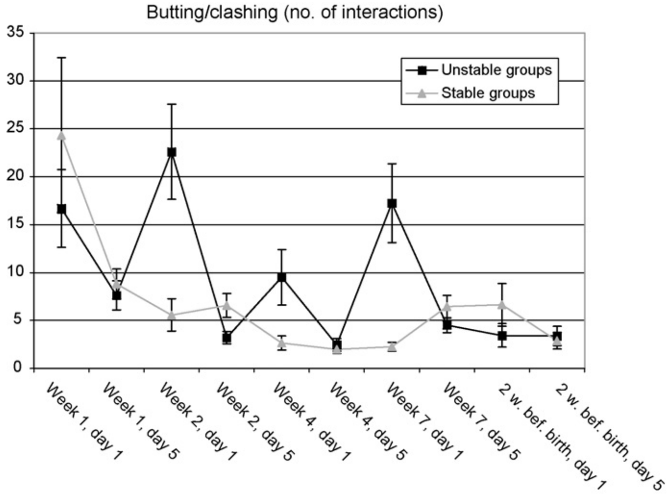

Fig. 3. Variation in butting/clashing activity (means ± S.E.) over time in goats from unstable and stable treatment groups. Day 1 is the day of rotating goats and first day of vide recording whereas day 5 is the last day of video recording in each experimental week.

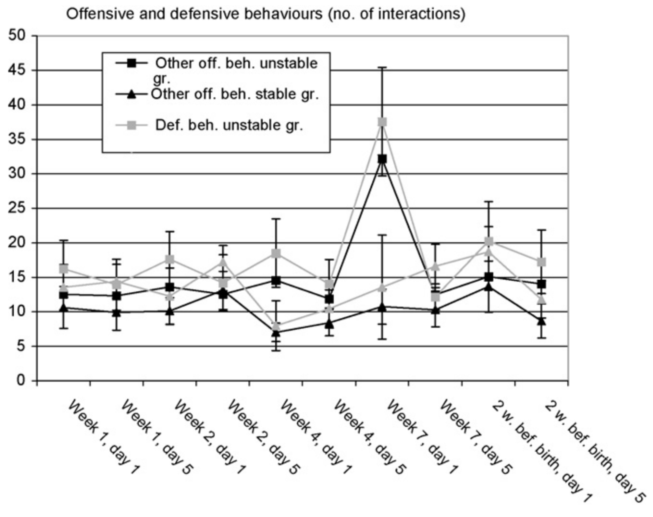

Fig. 4. Variation in number of offensive (butting/clashing excluded) and defensive behaviours (means ± S.E.) over time in goats from unstable and stable groups. Day 1 is the day of regrouping goats and first day of vide recording whereas day 5 is the last day of video recording in each experimental week.

($F_{1,58} = 6.0$, $P < 0.05$). In week 7 this difference between day 1 and 5 was significant for butting/clashing ($F_{1,58} = 3.8$, $P = 0.06$), defensive behaviours ($F_{1,58} = 5.2$, $P < 0.05$) and other offensive behaviours ($F_{1,58} = 5.2$, $P < 0.05$). Due to a significant interaction between treatment and day concerning butting/clashing activity, the effect of treatment was only significant on day 1 (week 2: $F_{1,58} = 13.8$, $P < 0.001$; week 4: ($F_{1,58} = 4.2$, $P < 0.05$, week 7: $F_{1,58} = 14.8$, $P < 0.001$).

In the first two weeks, the heavier goats showed more offensive (week 1: $F_{1,58} = 11.2$, $P < 0.01$; week 2: $F_{1,58} = 12.6$, $P < 0.001$) and less defensive behaviours (week 1: $F_{1,58} = 6.3$,

$P < 0.05$; week 2: $F_{1,58} = 9.6$, $P < 0.01$) than the lighter individuals. Age did not significantly affect any of the social behaviours.

For the unstable groups, butting/clashing activity was significantly less frequent in week 4 than in the other weeks on day 1 ($F_{4,8} = 3.1$, $P < 0.05$; Fig. 3). In the stable groups, the goats butted/clashed significantly more often at 6 (at the time of grouping) and 7 weeks and in the last part of pregnancy than in the rest of the experimental period ($F_{4,8} = 5.2$, $P < 0.001$). The amount of other offensive behaviours ($F_{4,8} = 2.8$, $P < 0.05$) and defensive behaviours ($F_{4,8} = 2.3$, $P = 0.07$) were both higher on the seventh grouping than on the other days of regrouping in the unstable groups whereas these behaviours did not change significantly with time in the stable groups (Fig. 4).

The variation over time (day 1 in week 1, 2, 4 and 7 and until 2 weeks before expected birth) in the relative number of offensive behaviours (butting/clashing included) by each individual was large and did not differ significantly between stable and unstable groups (unstable groups: CV ± S.E. = 93.3 ± 10.5, stable groups: CV ± S.E. = 78.9 ± 14.4). In the unstable groups, the largest range for one goat in butting/clashing activity was 1–62 initiated attacks between the first and last regrouping. In the stable groups, one goat varied from one date to the next with as much as 1–102 initiated attacks, which suggests that the degree of conflict is strongly dependent on the type of individuals that are grouped together even in the stable groups.

3.3. Growth in the goats

Twenty-one goats gained weight during the 7 weeks' experimental period, whereas 10 goats (five of which were from the stable groups and five of which were from the unstable groups) lost weight in the same period. There were no significant effects of treatment or age on the growth of the goats (means ± S.E., unstable groups: 1.2 ± 0.9 kg, stable groups: 0.9 ± 0.7 kg). The oldest goat, belonging to one of the stable groups, died in the middle of the experimental period before the last weighing.

3.4. Kid survival, sex and weight

Of the 31 goats, only two returned to oestrus during the experiment. Out of the 62 kids that were born, one was stillborn and only three of the live born kids died. Litter size was significantly larger for goats in the unstable groups than in the stable groups (mean ± S.E. unstable, $n = 16$: 2.2 ± 0.2; stable, $n = 13$: 1.9 ± 0.1; d$f = 1$, $\chi^2 = 7.9$, $P < 0.01$). The age and weight of the goats did not affect litter size significantly. In the twin litters ($n = 22$), there was no effect of treatment or weight of the mother on the sex ratio of the kids. However, 2- and 3 to 4-year-old goats gave birth to significant more FF litters than 5 to 9-year-old goats (d$f = 2$, $\chi^2 = 6.7$, $P < 0.05$). The proportion of females in the twin litters was 55 ± 15.7% in the unstable groups and 37.5 ± 10.9 in the stable groups.

Goats with a high mean level of cortisol throughout the experimental period, gave birth to significantly fewer kids ($R = -0.5$, $P < 0.01$), but there was no significant relationship between cortisol level and sex ratio in the litter.

There was no significant treatment effect on birth weight of the kids or on weight gain from birth until weaning, but males were heavier at birth (Lsmeans ± S.E. males: 3.1 ± 0.2 kg, females: 2.8 ± 0.2 kg; $F_{2,31} = 5.2$, $P < 0.05$) and gained more weight from birth until weaning than female kids (males: 8.6 ± 0.7 kg, females: 7.2 ± 0.7 kg; $F_{2,31} = 11.9$, $P < 0.01$). Neither birth weight nor growth were significantly affected by litter size, but there was a significant maternal effect on birth weight ($Z = 1.7$, $P < 0.05$) and growth of the kids ($Z = 2.7$, $P < 0.01$).

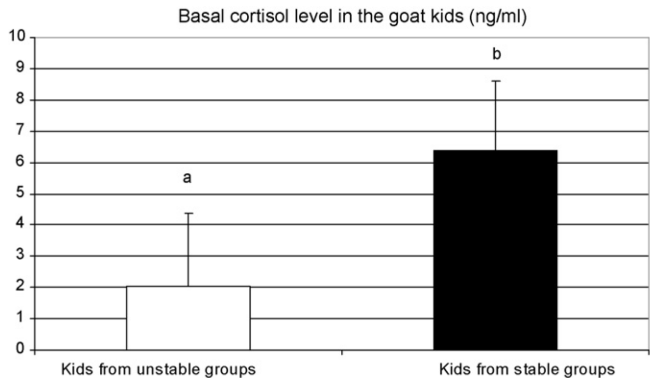

Fig. 5. Effect of the treatment on basal cortisol concentration (means ± S.E.) ng/ml in blood plasma of kids in the unstable and stable treatment groups. a, b: $P < 0.05$.

3.5. Basal cortisol concentration in the kids

Kids from stable groups had a significantly higher basal cortisol level than kids coming from goats in the unstable groups ($F_{1,22} = 4.79$, $P < 0.05$; Fig. 5). There were no significant effects of mother, sex of the kid or litter size on the level of cortisol.

3.6. Kid responses in the behavioural tests

Overall, only a few of the behavioural responses were affected by the treatment, and there were no significant differences between the sexes in any of the behaviours.

There were no significant effects of treatment on any of the behavioural variables measured in the novel object test, except that kids from goats kept in unstable groups tended to spend more time in contact with the novel object in their home pen when separated from their mother and social group than kids from goats kept in stable groups (unstable groups: 13.7 ± 1.9, stable groups: 9.0 ± 1.6; $F_{1,49} = 3.0$, $P = 0.09$). When separated from their group and confronted with a cage with two unfamiliar stimulus kids in their home pen, kids from the goats in the unstable groups tended to show more escape attempts from the pen at the age of 1 week than kids from the control goats (unstable groups: 2.9 ± 0.7, stable groups: 1.3 ± 0.4; $F_{1,49} = 2.9$, $P = 0.09$). However, this was not significant at 7 weeks of age. When conducting the same test 1 week after weaning at the age of 7 weeks, the kids from goats in the unstable groups made significantly more contacts with the cage in which new stimulus kids were placed ($F_{1,49} = 11.7$, $P < 0.01$; Fig. 6) than kids coming from goats in the stable social environment. Still, none of the other behavioural responses measured in this test differed significantly between treatments. Furthermore, kids from unstable groups showed a significantly larger decrease in number of escape attempts than kids from the control group from first to second social test ($F_{1,49} = 4.2$, $P < 0.05$; Fig. 7).

4. Discussion

As predicted, the aggression level was enhanced and much higher in the unstable than in the stable groups at all days of regrouping. Similar to what is documented in previous studies both in goats (e.g. Fernández et al., 2007) and cattle (e.g. Raussi et al., 2005), it appears that there is no habituation over time to the regrouping per se since the aggression level remains almost constant

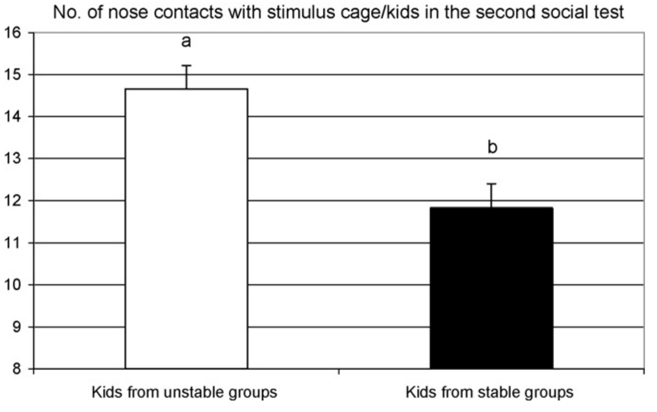

Fig. 6. Effect of the treatment on the number of nose contacts with the kids or cage (means ± S.E.) in kids from the unstable and stable treatment groups in the second social test. a, b: $P < 0.01$.

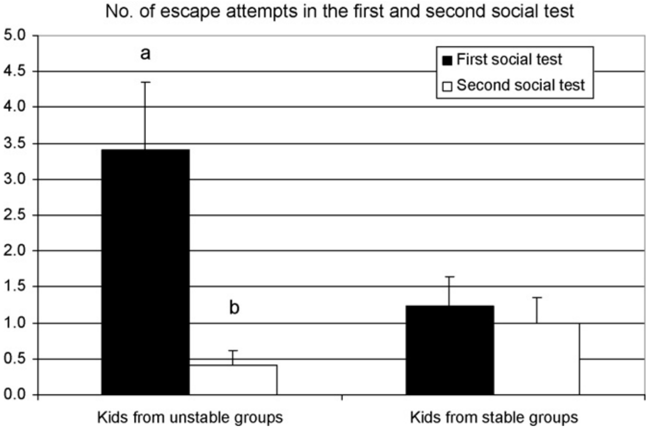

Fig. 7. Effect of the treatment on the number of escape attempts (means ± S.E.) in kids from the unstable and stable treatment groups in the first and second social test. a, b: $P < 0.05$.

at each regrouping. Meeting new group members will thus most certainly be associated with an increased aggression level. However, towards the end of each week of regrouping in the present experiment, the differences between unstable and stable groups were minimal. When mixing unfamiliar goats, the aggression level is reported to decline sharply already after 24 h (Alley and Fordham, 1994). Still, in several animal species it is documented that the intensive aggressive interactions that occur between unfamiliar individuals will affect access to important resources (Andersen et al., 1999; Andersen and Bøe, 2007; Bøe et al., 2006), reduce feed intake and growth (Stookey and Gonyou, 1994), lower the immune response (Tuchscherer et al., 1998; De Groot et al., 2001), reduce milk yield (Hasegawa et al., 1997; Fernández et al., 2007) and lower reproductive success (e.g. Mendl et al., 1992; Bakken, 1993a,b). Fernández et al. (2007) found negative effects on milk yield of goats at the first regrouping, but at the second and third regrouping the milk yield was no longer affected. This may suggest that the goats in some respect adapt to a social stress situation at least to the extent that the production level is no longer influenced.

The cortisol concentration did not differ significantly between goats from unstable versus stable groups in the present study. In the present study, the cortisol concentration measured the day after regrouping was very close to the level measured before the start of the experiment. This may suggest that the treatment only produced a moderate level of stress. Contrary to what was expected from other studies on regrouping and aggression in farm animals (e.g. Stookey and Gonyou, 1994), growth was not significantly affected in the present study, which may also indicate a moderate stress level. However, cortisol concentrations in goats and sheep are reported to decline rather quickly when they are repeatedly exposed to the same stressor over time (Roussel et al., 2004, 2008; Ropstad, unpublished). Thus, the fact that we did the first blood sampling on the day after rotation may be the most plausible explanation for not finding a significant difference in cortisol concentration between unstable and stable groups. It would have been beneficial to draw blood samples in the evening after finishing video recording behaviour. In goats, a sharp decline in cortisol can be seen 3–4 h after presentation of a stressor although the treatment continues for 8 h (Ropstad, unpublished). This also illustrates the difficulty in doing both behavioural observations and manually collecting physiological data simultaneously. Taking blood samples on the day of regrouping would probably increase the chance of getting a more reliable cortisol response, but to avoid interference with the behavioural observations, the samples would have to be collected after the behavioural observations. However, cortisol should normally be sampled during the morning. Finally, it is also possible that the handling during blood sampling per se is enough to produce an elevation in cortisol. The latter explanation may be strengthened by the fact that the variation in cortisol concentration in the goats from stable groups followed a similar pattern as what was found in the unstable groups. Although the handling time during each blood sampling in the present study was very short (these goats were used to blood sampling and handling before the start of this experiment), we still had to enter the pen and catch the goat. Irrespective of treatment, the cortisol concentration declined to a relatively low level in the last 2 weeks before birth of the kids, which suggests a low stress level at this time.

Studies of feral goat populations show that sheep and goats isolate themselves before, during and after parturition (e.g. reviewed by Shackleton and Shank, 1984), and that goats have a weak hiding phase lasting most commonly from 2 to 4 days after birth. After this initial hiding phase, the goat kids become followers and remain closely attached to their mother. Studies on activity patterns and companion preferences of goat kids, show that they start to form subgroups with other kids 7 days after birth, and by the end of the second week, the kids are closely associated with one another in peer groups during both activity and resting periods (Lickliter, 1984). We thus expected the kids to become increasingly motivated to get acquainted with other kids from around day 7, which was why we chose to run the first social test at the age of 1 week. Although few of the variables measured in the behavioural tests were significantly affected by the treatment, the most interesting findings were that the kids from the unstable groups spent more time in contact with a novel object, made more escape attempts in the first social test, and contacted the stimulus kids significantly more frequent in the second 'social test'. Furthermore, they also showed a larger decrease in escape attempts from the first to the second 'social test', indicating a lower fear level at the second trial and thus a higher rate of habituation. Although further research is needed to validate these tests, the kids from the unstable groups did show some signs of being more alert (i.e. more active escape attempts in the initial social test), more exploratory (i.e. more time in contact with a novel object and more contacts with the stimulus kids), and more flexible and easily habituated (i.e. larger reduction in escape attempts from trial one to trial two of the social test) when separated from their mother and the rest of the group in their home pen than the kids from the stable groups. Similar results have been found with other stressors in other species (e.g. rodents: Barbazanges et al., 1996; blue fox cubs: Braastad et al., 1998; lambs: Roussel et al., 2004 and goat kids: Roussel et al., 2005). However, in contrast to these studies, most of the experimental work on prenatal social stress in mice and rats documents higher levels of anxiety and lower locomotion score both in open field tests and novel object tests (Keeley, 1962; Lieberman, 1963; Batuev et al., 2000). Stressors used in rodents tend to be quite severe compared to the ones used in other species. Overall, when going through the literature, it becomes obvious that the results go in different directions. Thus, further research on prenatal stress in farm animals should focus more on the timing, duration as well as magnitude and type of the stressor used, since this may strongly affect whether the consequences are adaptive or not. Since goats, as for many other farm animals, are used to living under conditions with a relatively high animal density always involving a certain level of social stress, it may be beneficial to produce offspring that have a behavioural characteristic that allow them to cope with social challenges. These types of offensive, competitive offspring are also reported in high-density populations of wild species, such as the guinea pig (e.g. Rood, 1972; Asher et al., 2004). Social instability is in fact quite common even in the social life of wild mammals, since fluctuation from high to low population densities is more or less a general phenomenon (e.g.; Karels and Boonstra, 2000; Huitu et al., 2003; reviewed by Kaiser and Sachser, 2005).

Interestingly, being subjected to social instability during 7 weeks of gestation did not affect maternal cortisol, but still resulted in a decreased postnatal cortisol concentration in the offspring which was associated with different behavioural responses in the test situations than what was found in the kids from the stable groups. The higher basal cortisol level in the kids from stable groups in the present experiment is in great contrast to the results of Kaiser and Sachser (2001) on guinea pigs that were either subjected to a stable or an unstable social environment throughout pregnancy, and where the pups from the unstable environment showed a significantly higher cortisol concentration at a similar age as our goat kids. However, a major difference between these two studies was that the unstable groups of goats were regrouped for 7 weeks out of 5 months pregnancy (second trimester) while the guinea pigs were regrouped throughout the entire pregnancy. Previous work on rat pups (Takahashi and Kalin, 1991; Weinstock et al., 1998), lambs (Roussel et al., 2004) and goat kids (Duvaux-Ponter et al., 2003), where stressors such as transport and isolation were used, also document an increase in the basal cortisol level for the prenatally stressed offspring early in the postnatal period. Comparatively, Roussel et al. (2005) found no effect of transport stress in the last third of pregnancy on basal cortisol concentration of goat kids, but they did find an increased enzyme activity in one of the medullo-adrenals. Nevertheless, in accordance with our results, Kanitz et al. (2003) and Otten et al. (2001) found that piglets born to sows stressed by restraint during the last third of pregnancy, had a lower basal cortisol concentration than controls. Furthermore, Duvaux-Ponter et al. (2003) also documented a lower basal cortisol concentration in prenatally stressed goat kids at 2 days of age.

The higher basal concentration of cortisol in kids from the stable groups of the present experiment is difficult to explain, but it is important to be aware that stable groups may imply a relatively high level of social stress if some group members continue to fight over time. This questions the criteria for social stability and what really is the optimal social environment. Furthermore, kids coming from unstable maternal environments may be more adapted to a stress-situation and thus have a lower basal level of cortisol as suggested by the 'environment-adaptation' hypothesis. Overall, these results underpin the importance of investigating the impact of the social environment through a more functional approach as well as finding out what part of the physiological machinery that is most affected in the different species. In some cases the sympatho-adrenomedullary system may be more affected than the HPA-axis, and adrenal activity could thus be a more reliable measure than cortisol.

Except for the higher aggression level in the unstable groups, social instability did not have any other significant effects on factors such as growth, cortisol level or kid production in the goats. Similarly, there were only minor effects on the behavioural development in the goat kids. However, some of the results suggested a more active coping style in the kids from goats belonging to the unstable groups.

Acknowledgements

The project was funded by the Norwegian Research Council and 'Fondet for jordbruksmidler'. We thank Agnes Klouman, Kari Eikanger and Ellen Dahl for excellent technical assistance. MSc student Tone Urne also did a great job in collecting behavioural data and weighing the kids.

References

Alley, J.C., Fordham, R.A., 1994. Social events following the introduction of unfamiliar does to a captive feral goat (*Capra hircus* L.) herd. Technical note. Small Ruminant Res. 13, 103–107.

Andersen, I.L., Bøe, K.E., 2007. Resting pattern and social interactions in goats—the impact of size and organisation of lying space. Appl. Anim. Behav. Sci. 108, 89–103.

Andersen, I.L., Bøe, K.E., Kristiansen, A.L., 1999. The influence of different feeding arrangements and food type on competition at feeding in pregnant sows. Appl. Anim. Behav. Sci. 65, 91–104.

Asher, M., Oliviera, E.S., Sachser, N., 2004. Social system and spatial organization of wild guinea pigs (*Cavia aperea*) in a natural low density population. J. Mammal. 85, 788–796.

Bakken, M., 1993a. Reproduction in farmed silver fox vixens, *Vulpes vulpes*, in relation to own competition capacity and that of neighbouring vixens. J. Anim. Breed Genet. 110, 305–311.

Bakken, M., 1993b. The relationship between competition capacity and reproduction in farmed silver fox vixens (*Vulpes vulpes*). J. Anim. Breed Genet. 110, 147–155.

Barbazanges, A., Piazza, P.V., Le Moal, M., maccari, S., 1996. Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. J. Neurosci. 16, 3943–3949.

Barroso, F.G., Alados, C.L., Boza, J., 2000. Social hierarchy in the domestic goat: effect on food habits and production. Appl. Anim. Behav. Sci. 69, 35–53.

Batuev, A.S., Poliakova, O.N., Aleksandrov, A.A., 2000. The effect of 'social stress' during rat pregnancy on the anxiety level of the progeny. Zh Vyssh Nerv Deiat Im IP Pavlova 50, 281–286.

Bøe, K.E., Berg, S., Andersen, I.L., 2006. Resting behaviour and displacements in ewes—effects of reduced lying space and pen shape. Appl. Anim. Behav. Sci. 98, 249–259.

Boivin, X., Braastad, B.O., 1996. Effects of handling during temporary isolation after early weaning on goat kids' later response to humans. Appl. Anim. Behav. Sci. 48, 61–71.

Braastad, B.O., 1998. Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. Appl. Anim. Behav. Sci. 61, 159–180.

Braastad, B.O., Osadchuk, L.V., Lund, G., Bakken, M., 1998. Effects of prenatal handling stress on adrenal weight and function and behaviour in novel situations in blue fox cubs. Appl. Anim. Behav. Sci. 57, 157–169.

Carson, K., 1985. Exploratory behaviour. In: Fraser, A.F. (Ed.), Ethology of Farm Animals. A Comprehensive Study of the Behavioural Features of the Common Farm Animals. Elsevier, pp. 201–207.

Côté, S.D., Festa-Bianchet, M., 2001. Reproductive success in female mountain goats: the influence of age and social rank. Anim. Behav. 62, 173–181.

De Groot, J., Ruis, M.A.W., Scholten, J.W., Koolhaas, J.M., Boersma, J.A., 2001. Long-term effects of social stress on antiviral immunity in pigs. Physiol. Behav. 73, 145–158.

Duvaux-Ponter, C., Roussel, S., Tessier, J., Sauvant, D., Ficheux, C., Boissy, A., 2003. Physiological effects of repeated transport in pregnant goats and their offspring. Anim. Res. 52, 553–566.

Færevik, G., Jensen, M.B., Bøe, K.E., 2006. Dairy calves social preferences and the significance of a companion animal during separation from the group. Appl. Anim. Behav. Sci. 99, 205–221.

Fernández, M.A., Alvarez, L., Zarco, L., 2007. Regrouping in lactating goats increases aggression and decreases milk production. Small Ruminant Res. 70, 228–232.

Fournier, F., Festa-Bianchet, M., 1995. Social dominance in adult female mountain goats. Anim. Behav. 49, 1449–1459.

Hasegawa, N., Nishiwaki, A., Sugawara, K., Ito, I., 1997. The effects of social exchange between two groups of lactating primiparous heifers on milk production, dominance order, behaviour and adrenocortical response. Appl. Anim. Behav. Sci. 51, 15–27.

Hatcher, L., Stepanski, E.J., 1994. A Step-by-Step Approach to Using the SAS® System for Univariate and Multivariate Statistics. SAS Institute Inc., Cary, NC, 552 pp.

Huitu, O., Norrdahl, K., Korpimaki, E., 2003. Landscape effects on temporal and spatial properties of vole population fluctuations. Oecologia 135, 209–220.

Jarvis, S., Moinard, C., Robson, S.K., Baxter, E., Ormandy, E., Douglas, A.J., Seckl, J.R., Russell, J.A., Lawrence, A.B., 2006. Programming the offspring of the pig by prenatal social stress: neuroendocrine activity and behaviour. Horm. Behav. 49, 68–80.

Jørgensen, G.H.M., Andersen, I.L., Bøe, K.E., 2006. Feed intake and social interactions in dairy goats—the effects of feeding space and type of roughage. Appl. Anim. Behav. Sci. 107, 239–251.

Kaiser, S., Sachser, N., 2001. Social stress during pregnancy and lactation affects in guinea pigs the male offspring's endocrine status and infantilizes their behaviour. Psychoneuroendocrinology 26, 503–519.

Kaiser, S., Sachser, N., 2005. The effects of prenatal social stress on behaviour: mechanisms and function. Neurosci. Biobehav. Rev. 29, 283–294.

Kanitz, E., Otten, W., Tuchscherer, M., Manteuffel, G., 2003. Effects of prenatal stress on corticosteroid receptors and monoamine concentrations in limbic areas of suckling piglets (*Sus scrofa*) at different ages. J. Vet. Med. Ser. A 50, 132–139.

Karels, T.J., Boonstra, R., 2000. Concurrent density dependence and independence in populations of arctic ground squirrels. Nature 408, 460–463.

Keeley, K., 1962. Prenatal influence on behaviour of offspring of crowded mice. Science 135, 44–45.

Kongsted, A.G., 2006. relation between reproduction performance and indicators of feed intake, fear and social stress in commercial herds with group-housed non-lactating sows. Livest. Sci. 101, 46–56.

Lickliter, R.E., 1984. Hiding behavior in domestic goat kids. Appl. Anim. Behav. Sci. 12, 245–251.

Lieberman, M.W., 1963. Early developmental stress and later behaviour. Science 141, 824–825.

Mendl, M., Zanella, A.J., Broom, D.M., 1992. Physiological and reproductive correlates of behavioural strategies in female domestic pigs. Anim. Behav. 44, 1107–1121.

Otten, W., Kanitz, E., Tuchscherer, M., Nürnberg, G., 2001. Effects of prenatal restraint stress on hypothalamic–pituitary–adrenocortical and sympatho-adrenomedullary axis in neonatal pigs. Anim. Sci. 73, 279–287.

Raussi, S., Boissy, Delval, E., Pradel, P., Kaihilahti, Veissier, I., 2005. Does repeated regrouping alter the social behaviour of heifers? Appl. Anim. Behav. 93, 1–12.

Rood, J.P., 1972. Ecological and behavioural comparisons of three genera of Argentine cavies. Anim. Behav. Mon. 5, 1–83.

Roussel, S., Hemsworth, P.H., Boissy, A., Duvaux-Ponter, C., 2004. Effects of repeated stress during pregnancy in ewes on the behavioural and physiological responses to stressful events and birth weight of their offspring. Appl. Anim. Behav. Sci. 85, 259–276.

Roussel, S., Boissy, A., Montigny, D., Hemsworth, P.H., Duvaux-Ponter, C., 2005. Gender-specific effects of prenatal stress on emotional reactivity and stress physiology of goat kids. Horm. Behav. 47, 256–266.

Roussel, S., Hemsworth, P.H., Boissy, A., Duvaux-Ponter, C., 2008. Repeated transport and isolation during pregnancy in ewes. II. Differential effects on emotional reactivity and weight of the offspring and the responses of the ewes to the stressors. Appl. Anim. Behav. Sci. 109, 275–291.

SAS Institute Inc., 1989. SAS/STAT® User's Guide, Version 6, Fourth ed., vol. 1. SAS Institute Inc, Cary, NC, p. 943.

Shackleton, D.M., Shank, C.C., 1984. A review of the social behaviour of feral and wild sheep and goats. J. Anim. Sci. 58, 500–509.

Shank, C.C., 1972. Some aspects of social behaviour in a population of feral goats (*Capra hircus* L.). Z. Tierpsychol. 30, 488–528.

Simensen, E., Olson, L.D., Vanjonack, W.J., Johnson, H.D., Ryan, M.P., 1978. Determination of corticosterone concentration in plasma of turkeys using radio-immunoassay. Poult. Sci. 57, 1701–1704.

Stookey, J.M., Gonyou, H.W., 1994. The effects of regrouping on behavioral and production parameters in finishing swine. J. Anim. Sci. 72, 2804–2811.

Takahashi, L.K., Kalin, N.H., 1991. Early developmental and temporal characteristics of stress-induced secretion of pituitary-adrenal hormones in prenatally stressed rat pups. Brain Res. 558, 75–78.

Tuchscherer, M., Puppe, B., Tuchscherer, A., Kanitz, E., 1998. Effects of social status after mixing on immune, metabolic, and endocrine responses in pigs. Phys. Behav. 64, 353–360.

Weinstock, M., Poltyrev, T., Schorer-Apelbaum, D., Men, D., Mccarty, R., 1998. Effect of prenatal stress on plasma corticosterone and catecholamines in response to footshock in rats. Physiol. Behav. 64, 439–444.